

IN THE SPECIFICATION:

In line 3, page 1, please insert the following:

-- This application is a divisional of U.S. Serial No. 09/665,819, filed September 20, 2000, which claims the benefit of priority from U.S. Provisional application No. 60/155,068, filed September 21, 1999. --

Amend page 11, line 19, to page 12, line 4, as follows:

Figure 3 illustrates the JKAP amino acid sequence analysis and expression pattern. (A) Amino acids sequences corresponding to the catalytic domains of selected MAPK phosphatases were aligned in PIMA 1.4 using sequential branching clustering. The resulting alignment was processed in BOXSHADE. Sequence identities are highlighted in black; similarities are highlighted in gray. Gaps are indicated by dots. The aligned sequences include mJKAP (SEQ ID NO: 5); puckered (SEQ ID NO: 6); rMKP-3 (SEQ ID NO: 7); rMKP-X (SEQ ID NO: 8); hMKP-4 (SEQ ID NO: 9); rMKP-2 (SEQ ID NO: 10); mMKP-1 (SEQ ID NO: 11); mH3/6 (SEQ ID NO: 12); mPAC-1 (SEQ ID NO: 13); hVH3 (SEQ ID NO: 14); and hVHR (SEQ ID NO: 15) and the resulting consensus sequences (SEQ ID NO: 16 to 20). (B) Phylogenetic analysis was carried out on aligned sequences by parsimony in PHYLIP using PROTPARS with bootstrapping of 1000 replicates. The numbers correspond to the occurrences of the branch in the consensus tree. (C) Polyadenylated mRNA from adult mouse tissues was hybridized with a JKAP cDNA probe. mRNA integrity and quantity was confirmed by hybridization with β -actin. Molecular weights in kilobase pairs are indicated on the left.

Amend page 69, line 7 to 25, as follows:

To facilitate rapid isolation of novel sequence extending the known LS20 sequence, anchored PCR was used on these 13 primary plaque pools. Three synthetic oligonucleotides were prepared. The first oligonucleotide, 1065-30 (SEQ ID NO: ~~17~~ 21), being identical in sequence to a 32 nucleotide region of the left lambda phage vector arm and flanking on one side the insert cloning site within the vector:

~~(5'-CCTTTTGTGAGCAAGTTCAGCCTGGTTAAGTCC-3') (SEQ ID NO:17)~~

(5'-ccttttgagcaagttcagcctggttaagtcc-3') (SEQ ID NO:21).

The second oligonucleotide, 1386-58 (SEQ ID NO: ~~48~~ 22), being identical in sequence to a 33 nucleotide string near the 5'-end of the LS20-2 insert:

~~(5'-GGAGGCCTCTCTCTGTGTGTGTGGAGCCCTCAGG-3') (SEQ ID NO:22)~~

(5'-ggaggcctctctctgtgtgtgtggagccctcagg-3') (SEQ ID NO:22);

The third oligonucleotide, 1386-59 (SEQ ID NO: ~~49~~ 23), being complementary (anti-sense) to a 31 nucleotide string near the 3'-end of the LS20-2 insert:

~~(5'-GGCAGCACCAGCCTGAACTTTGCAATATTTTC-3') (SEQ ID NO:19)~~

(5'-ggcagcaccagcctgaactttgcaatatttc-3') (SEQ ID NO:23).

Amend page 71, line 2-18, as follows:

A FASTA search of Genbank EST sequences with the novel mouse LS20 cDNA sequence revealed a high homology hit with a human EST designated clone 249002. The clone 249002 was purchased from an IMAGE consortium supplier (Genome Systems, St. Louis, MO) and sequenced in its entirety. The insert was short, 614 bp. The insert of clone 249002 was isolated by digestion with the restriction enzymes EcoRI and NotI, gel-purified and used as a template for the synthesis of random hexamer-primed probes used in subsequent screens of a human fetal liver cDNA library (Clontech). In addition, two new oligonucleotide primers were synthesized based on the human LS20 sequence. Sense (1470-25) (SEQ ID NO: ~~20~~ 24) and anti-sense (1470-26) (SEQ ID NO: ~~21~~ 25) primers were designed to allow amplification of a 143bp internal fragment of the human LS20 sequence or for use in an anchored PCR scheme similar to that used in the cloning of the mouse LS20 cDNA described above.

~~(5'-CAGCAGCGG-ATTCACCATC-3') (SEQ ID NO:20)~~

~~(5'-GCGATCACCAGTGTACGCG-3') (SEQ ID NO:21)~~

(5'-c agcagcgg attcaccatc-3') (SEQ ID NO:24)

(5'-gcgatcaccagtggtcacgc-3') (SEQ ID NO:25)

Amend page 76, line 21 to page 78, line 2, as follows:

Given the close sequence similarity of JKAP to MAPK phosphatases, the activity of JKAP phosphatase in MAPK cascades was examined. We assayed MAPK activity in 293T cells co-transfected with the JKAP phosphatase and either JNK1, ERK2, or p38 using an immunocomplex kinase assay. Endogenous JNK1 and over-expressed HA-JNK1 were immunoprecipitated by incubation with rabbit anti-JNK1 polyclonal antibody (Ab101) and mouse anti-HA monoclonal antibody (12CAS), respectively, plus protein A-agarose beads (Bio-Rad) in lysis buffer (20 mM HEPES, pH 7.4, 2mM EGTA, 50 mM glycerophosphate, 1% Triton X-100, 10% glycerol, 1mM dithiothreitol, 2 µg/mL aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1mM NaCl and 1 mM Na₃VO₄). The precipitates were washed twice with lysis buffer, twice with LiCl buffer (500 nM LiCl, 100 mM Tris-HCl, pH 7.6 and 0.1% Triton X-100), and twice with kinase buffer (500 mM LiCl, 100 mM Tris-HCl, pH 7.6, and 0.1% Triton X-100), and twice with kinase buffer (20 mM MOPS, pH 7.2, 2 mM EGTA, 10 mM MgCl₂, 1mM dithiothreitol, 0.1% Triton X-100, and 1 mM Na₃VO₄). The pellets were then mixed with 1 µg of GST-clun (1-79), 15 µM ATP, and 10 µgCi of [³²P] ATP in 30 µL of kinase buffer. The kinase reaction was performed at 30 C for 30 min. and terminated with an equal volume of SDS sampling buffer. The reaction mixtures were analyzed by SDS-PAGE and autoradiography. Polyclonal Ab101 was derived from rabbits that were immunized with peptide

N'- [[- -]] CKNGVIRGQPSPLAQVQQ [[- -]] -C' (SEQ ID NO:23 27)

The carrier used was KLH. This antibody was prepared by standard methods using two injections and termination three weeks after burst injection. One suitable source for preparation of Ab101 (titer >1:10K) is Genemed Synthesis, Inc See also Chen, Y.-R., Meyer, C.F., and Tan, T.-H., (1996). Persistent activation of c-Jun N-terminal kinase 1(JNK1) in gamma radiation-

induced apoptosis. J.Biol. Chem. 271:631-634; Hu, M. C.-T., Qiu, W.R, Wang, X., Meyer, C.F, and Tan, T.-H., (1996). Human HPK1, a novel human hematopoietic progenitor kinase that activates the JNK/SAPK kinase cascade. Gene & Development 10:2251-2264.; Wang W., Zhou, G., Hu, M.C.-T., Yao, Z., and Tan,T.-H., (1999). Activation of Hematopoietic progenitor kinase 1 (HPK1)-dependent, stress-activated c-Jun N-terminal kinase (JNK) pathway by transforming growth factor beta (TGF-beta)-activated kinase (TAK1), a kinase mediator of TGF-beta signal transduction. J. Biol. Chem. 272:22771-22776; Ensenat, D., Yao Z., Wang X.S., Kori, R., Zhou, G., Lee, S.C, and Tan, T.-H., (1999). A novel Src homology 3 domain-containing adaptor protein, HIP55, that interacts with hematopoietic progenitor kinase 1. J. Biol. Chem. 274:33945-33950; Zhou, G., Lee, S.C, Yao, Z., and Tan,T.-H., (1999). Hematopoietic progenitor kinase 1 is a component of transforming growth factor beta-induced c-Jun N-terminal kinase signaling. J. Biol. Chem. 274:13133-13138.

Amend page 82, line 10-18, as follows:

In this experiment, a peptide containing sequence of 5' end of human JNK activating phosphatase (SEQ ID NO: ~~22~~ 26) was generated and used for antibody induction. This peptide was coupled with carrier protein keyhole limpet hemacyanin and injected into rabbits at a boost schedule of once every two weeks. Titer of antibody against the peptide was determined using ELISA assays with plates immobilized with the peptide. One of the animals (#1436) was determined to have the highest titer against the peptide.

~~H2NH2~~N-CGNFKDARDAEQLS-COOH (SEQ ID NO: ~~22~~ 26)

Please replace the Sequence Listing pages 84-92 with the attached substitute expanded Sequence Listing pages 84-100.

Please renumber pages 93-97 as new pages 101-105.